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ORIGINAL ARTICLE *Inhibitors*

Non-genetic risk factors and the development of inhibitors in haemophilia: a comprehensive review and consensus report

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Summary. The development of inhibitors to the infused factor in patients with haemophilia is a serious clinical problem. Recent evidence suggests that alongside the strong genetic contribution to inhibitor formation, there are a number of non-genetic factors – perceived by the immune system as danger signals – which promote formation of inhibitors. This study provides a comprehensive review of clinical studies relating to these factors and also presents a survey of opinion concerning their importance and clinical influence, conducted among the members of the European Haemophilia Treatment Standardisation Board (EHTSB). Taken together, this information highlights the lack of robust data concerning the influence of several non-genetic risk factors on inhibitor development, and an urgent need for prospective, well-conducted studies that adhere to recommendations made by the European Medicines Agency (EMEA) for studying inhibitors. Based on current literature, the EHTSB formulated consensus recommendations. It is desirable to minimize intensive treatment wherever possible, given the clinical situation. Prophylaxis should be offered to all children, although we still need to determine optimal dosing with respect to inhibitor development, and age for starting treatment. Vaccinations should be given subcutaneously and concomitant factor concentrate infusions avoided. According to the board, there is no evidence in the literature supporting suggestions that the type of concentrate influences inhibitor risk; but all patients should be monitored during their first exposures. Furthermore, there is no evidence to support an association between pregnancy-related issues, breast feeding and treatment-related factors (e.g. route of administration, or use of blood components) and inhibitor development.

Keywords: EHTSB, haemophilia, inhibitors, non-genetic, risk factors

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Introduction

A major challenge in the treatment of people with haemophilia is the development of neutralizing anti-factor VIII (FVIII) and factor IX (FIX) antibodies

(inhibitors) that compromise the activity of the administered factor [1,2]. The appearance of these inhibitors in the circulation is the outcome of a multi-step process that involves a cascade of interactions between different cells of the innate and adaptive immune system in very distinct compartments. Each step in this cascade is tightly regulated by stimulatory and inhibitory signals that determine the activation state of the immune cells involved and their migration into distinct lymphoid compartments [3]. Any event that alters the balance between the signals will have the potential to modulate these steps, and the development of inhibitory antibodies is therefore most likely determined by a close interaction between different risk factors or events. The activation of $CD4^+$ T cells that help B cells to differentiate into antibody producing plasma cells requires an effective interaction with antigen-presenting cells that present FVIII or FIX peptides in the context of MHC-class II. The effectiveness of this interaction depends on the maturation state of the antigen-presenting cells. This is influenced by genetic factors determining the sensitivity of the innate immune system to respond to certain immune stimuli and by the local environment that provides the immune stimuli. In recent years, stimuli of the innate immune system have been named 'danger signals'. Today, it is well established that danger signals can arise from both exogenous and endogenous sources [4]. Typical exogenous sources are microbial agents that trigger toll-like receptors and other microbial sensors [5,6]. Therefore, any infection and certain vaccinations that occur at the time of treatment with the deficient factor should be considered as potential risk factors for the development of inhibitors. Endogenous sources of danger signals are mostly associated with tissue damage that involves necrotic cell death. Cells that die by necrosis release endogenous danger signals that stimulate the innate immune system [4]. Severe bleeds and surgery are most likely to be associated with such necrotic cell damage and could, therefore, contribute to the risk for a patient to develop inhibitors. One way to avoid necrotic cell damage at the time of treatment would be to administer the factor during bleeding-free intervals. For clinical reasons this is not always possible, yet prophylactic treatment of patients might well impose a lower risk than on-demand treatment [7].

Several findings during the last decade clearly indicate that genetic factors are major determinants of the outcome. However, the influence of non-genetic factors related to patients and treatment is also appreciated and will likely, in many cases, be decisive. Therefore, the better we understand the impact of each potential risk factor and danger signal, the better able we will be to identify the determinants of risk for an individual patient in a particular situation, and optimize management in the clinical setting. To shed some light on the importance of non-genetic candidates for inhibitor risk, the European Haemophilia Therapy Standardisation Board (EHTSB) – a network of haemophilia physicians in Europe – reviewed the current literature on the risk factors which have the potential to generate danger signals for the innate immune system. The risk factors assessed were divided into five groups: (i) pregnancy/delivery issues and breast feeding, (ii) age at start of treatment, reason for first infusion and prophylactic vs. on-demand treatment, (iii) vaccinations, infections, extravascular infusions, blood components, concurrent immunological disorders, (iv) severe bleeds, intensity of treatment, surgery and continuous vs. bolus infusions, and (v) type of factor concentrate.

Besides providing a comprehensive review of the literature, the study also reports on a survey of clinical practice among the EHTSB centres in Europe. Consensus statements and treatment recommendations are provided reflecting the European Medicines Agency (EMEA) guidelines [8], the literature and current practice.

Methods

Literature review

The literature search was carried out in May 2008, and updated in January 2010, using the PubMed database. The terms used were 'Hemophilia/haemophilia A/immunology'[MeSH] OR 'Hemophilia/haemophilia B/immunology'[MeSH]) OR 'Factor VIII/antagonists and inhibitors'[MeSH] OR 'Factor VIII/immunology'[MeSH] OR 'Factor IX/antagonists and inhibitors'[MeSH] OR 'Factor IX/immunology'[MeSH]. Further selection of appropriate studies was carried out manually by the authors. Case–control studies, cohort studies and case series were included, but single case reports and abstracts were excluded.

All data were extracted from the articles by 2–4 authors and classified according to the following definitions: a cohort study was defined as a longitudinal follow-up of a group of unselected patients with known risk factors to evaluate the outcome/inhibitor development at the conclusion of the study. A case–control study was defined as a cross sectional study that included a number of patients with inhibitors (cases) and another group of (matched) patients without inhibitors (controls). Specified risk factors

for inhibitor development were then analysed in both groups. Case series were defined as a longitudinal follow-up of a group of patients selected for certain risk factors to evaluate the outcome/inhibitor development at the end of the observation period.

Extracted data were used to populate a standard form. Items included: study design; number of patients in the study; patient characteristics (severity of haemophilia, treatment status); inhibitor testing (frequency, assay used and cut-off level); treatment characteristics (type of product); analysis of the risk factor [relative risk (RR), hazard ratio, odds ratio (OR) or otherwise, as stated by the authors]. If no RR or OR was given these measures were calculated whenever possible, using the available data in the article.

EHTSB survey

The EHTSB is an established group of internationally recognized European experts in the field of haemophilia and blood clotting disorders. Founded in 2003 by Baxter, the board currently represents 24 large European haemophilia centres in 15 countries, taking care of >4000 patients with severe congenital bleeding disorders from birth to adulthood. In conjunction with the literature review, a survey was undertaken to assess all members' opinions of the importance of risk factors on the development of inhibitors and how this influenced their clinical practice. In a subgroup of 14 EHTSB members, the potentially most important factors involved in inhibitor development were discussed and listed. Based on this risk factor selection, two questionnaires were prepared and administered to all 24 EHTSB members. In the first questionnaire, board members were asked to rank each risk factor on a scale of 0–5 (0 = not important or not influential; 5 = very important or very influential) for importance of its potential role in inhibitor development. In the other questionnaire, the influence of the same single factors on their clinical practice was rated on a scale of 0–100.

The consensus recommendations were formulated following a discussion held within the subgroup of 14 members during an EHTSB meeting in Brussels on 15–16 January, 2009 and reviewed after the literature update in 2010.

Results

Pregnancy/delivery issues and breast feeding

Antenatal exposure to maternal FVIII, and breast feeding, has been considered potentially protective against inhibitor development [9]. Supporting this hypothesis is the fact that human breast milk affects normal gastrointestinal development and oral immune tolerance [10]. Moreover, the presence of fat globule proteins in breast milk that bear strong homology with FVIII might facilitate immune tolerance in the immature neonatal system, thus decreasing the likelihood of inhibitor formation [9,11].

Five studies were identified for review (Table 1) [12–16]. Two examined breastfeeding exclusively; two considered a variety of antenatal and perinatal risk factors (e.g. amniocentesis, villocentesis, premature birth and caesarean section) and one recent case–control study evaluated breast feeding as one of the potential risk factors [16]. No association could be found between breastfeeding and inhibitor development in any of the studies. Furthermore, there was no support for an association of inhibitors with other pregnancy-related issues or premature birth. Weaknesses in these studies were that the duration of follow-up was variable and not clearly defined in each study and that confounding factors were not taken into account.

Survey. These findings were in agreement with the survey results from the board members, the majority of whom rated pregnancy and delivery issues and breast feeding of none, very low or low importance (0–2) in clinical practice (Figs 1 and 2).

Recommendations. There are no data in the literature indicating an association between inhibitor formation and pregnancy-related issues, mode of delivery or breastfeeding. The board, therefore, made no recommendations regarding these topics for the purpose of reducing inhibitor incidence.

Age at start, reason for first infusion and prophylactic vs. on-demand FVIII treatment

Today, children with haemophilia can look forward to a favourable orthopaedic outcome and a good health-related quality of life. However, the age at which to initiate therapy and how to start treatment is still a matter of debate. It is difficult to isolate the age at first exposure to the deficient factor as a risk factor for inhibitor development. Seven studies were located that addressed these issues [13,15,17–21]. Two earlier studies [17,18] (Table 2) focused exclusively on age and concluded that age at start of treatment was inversely correlated with the risk of developing antibodies against FVIII. Later studies, which considered confounding factors such as the inherited FVIII mutation and intensity of treatment,

Table 1. Influence of pregnancy/delivery issues and breast feeding on inhibitor development.

Study	Type of study No. patients	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Inhibitor incidence	OR/RR (95% CI)	Results and conclusions
Knobe *et al.*, 2002a [12]	Retrospective CH 116	<1% PUPs	BA and Malmö inhibitor assay (Cut-off NA) ≥2× per year	HA 19% HB 37%	Insufficient data to calculate	No difference in total time of breast feeding between patients with and without inhibitors (P = 0.22) No association with breastfeeding
Santagostino *et al.*, 2005 [13]	CC 108	<2% PUPs, MTPs	NMA and BA (>0.5 twice) At least 1× per 3 months for first 100 EDs 1× per 6 months 100–200 EDs 1× per year thereafter	NA*	BF ≤6 months: 1.6 (0.5–5.0) BF >6 months: 1.9 (0.6–6.4)	No association between breast feeding, amniocentesis, premature birth, caesarean section and inhibitors
Jansen *et al.*, 2005 [14]	Retrospective CH 90	Severe (NOS) PUPs	<1996 BA (>1.0) >1996 NMA (>0.3) Frequency NA	20% (21% Breast fed; 18% not breast fed)	1.2 (0.5–2.9)†	No association with breast feeding
Gouw *et al.*, 2007a [15]	Retrospective CH 132 (91 breast fed; 41 not breast fed)	<2% PUPs	BA and NMA (Cut-off NA) Frequency NA	24%	Crude RR 0.9 (0.5–1.7) Adjusted RR 1.3 (0.6–2.8)‡	No association with breast feeding
Ragni *et al.*, 2009 [16]	CC 77	All severities NA	NA (Cut-off NA) Frequency NA	NA*	0.6 (0.2–2.2)	No association with breastfeeding

BA, Bethesda assay; BU, Bethesda units; CC, case–control study; CH, cohort study; CS, case series; ED, exposure day; HA, haemophilia A; HB, haemophilia B; MTP, minimally treated patients; NA, not available; NMA, Nijmegen assay/modification; NOS, not otherwise specified; OR, odds ratio; PUP, previously untreated patients; pdFVIII, plasma derived FVIII; rFVIII, recombinant FVIII; OR, Odds ratio; RR, relative risk; BF, breastfeeding.

*Incidence is only applicable in cohort study, not in CC.

†Calculated by the current authors from data given in the cited study.

‡Adjusted for baseline factor VIII activity level, ethnicity, factor VIII gene mutation type, age at first exposure, duration between exposure days, dose, prophylaxis and product type.

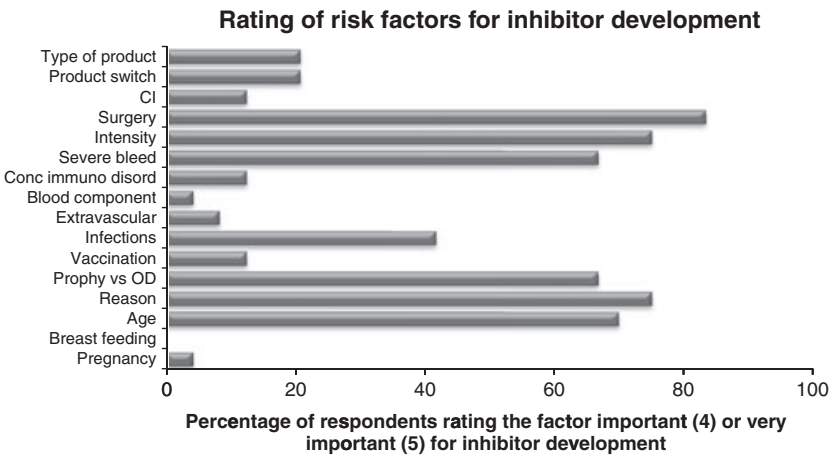

Fig. 1. Estimation of the importance of pregnancy and delivery issues, breast feeding, age at start of treatment, reason for first treatment and prophylactic vs. on-demand treatment, vaccinations, infections, extravascular infusions, blood components, concurrent immunological disorders, major bleeds, intensity of treatment, surgery, continuous infusion, product switch and product type on inhibitor formation. Twenty-four members of the European Haemophilia Therapy Standardisation Board completed the survey and the figure represents the percentage rating the factor important (4) or very important (5) for the development of inhibitors.

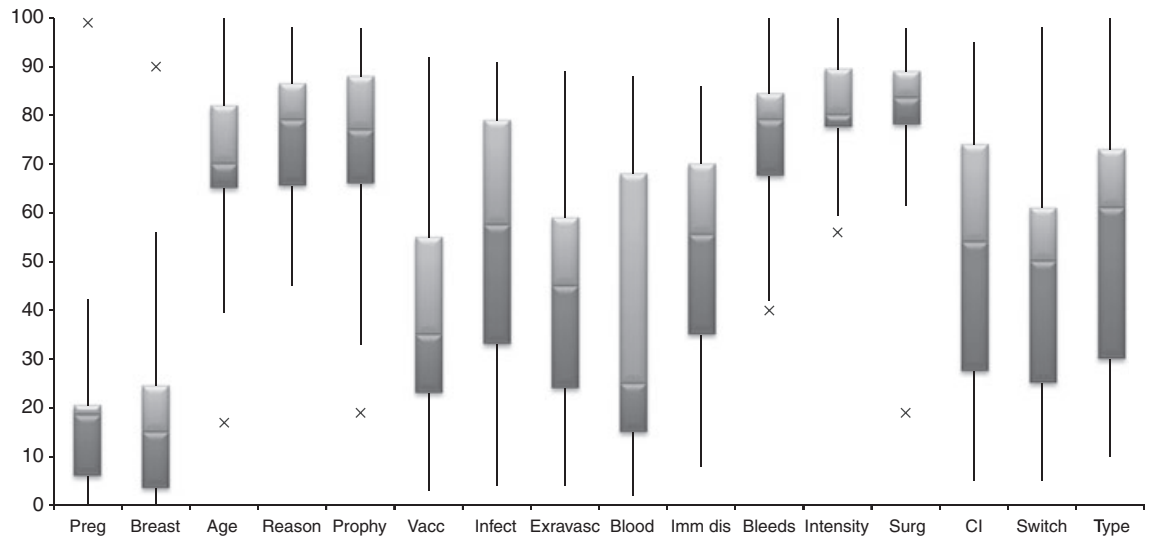

Fig. 2. Results of the survey of the European Haemophilia Therapy Standardisation Board group. Participants (n = 24) were asked to rate how each factor influenced their clinical practice on a scale of 0–100.

were unable to confirm this finding (Table 2) [13,15,20].

Several studies have evaluated prophylactic vs. on-demand treatment and, to a lesser extent, also attempted to analyse the reasons for the first infusion [13,15,19]. These studies, involving a total of 580 patients, indicate that prophylactic treatment might play a protective role against inhibitor development. In the recent study by Kurnik *et al.* [21], the dose at the start of prophylactic treatment was also suggested to be of importance. This study demonstrated that minimizing immunological danger signals during the first 20 EDs may reduce the risk of inhibitor formation.

Survey. The board members were in agreement that these factors were found to be reasonably important influences on the risk of inhibitor development. However, their influence on clinical practice was highly variable with only the use of prophylactic vs. on-demand treatment being rated of moderate to moderately high (3–4) significance (Figs 1and 2).

Recommendation. The EHTSB recommended that prophylaxis should be provided for all children to prevent bleeds. In addition, prophylaxis might exert a favourable immunological effect to promote tolerance. However, to better appreciate the immunological effect well-designed prospective studies are needed. This is also the case for evaluating the optimal dosing and when to start treatment. Haemophilia registries and large cohort studies can provide considerable insight. In all cases, however, the genetic aspects must be taken into account.

Table 2. Influence of age at start of factor replacement, reason for the first infusion and prophylactic vs. on-demand FVIII treatment on inhibitor development.

Study	Type of study No. patients	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Inhibitor incidence	OR/RR	Results and conclusions
Lorenzo *et al.*, 2001 [17]	Retrospective CH 62	<2% PUPs	BA (0.6) ≥1× per year	≤6 months: 41% 7–12 months: 29% >12 months: 12%	0.5 (0.27–0.92) (multivariate analysis performed by authors)†	Age at start of treatment appears to influence inhibitor development
van der Bom *et al.*, 2003 [18]	Retrospective CH 81	<1% PUPs	BA (1.0) ≥1× per year	<6 months: 34% 6–12 months: 20% 12–18 months: 13% >18 months: 0%	Hazard ratio 0.88 (0.77–0.99) for each month later of starting treatment	Age at first exposure is inversely associated with the risk of inhibitor development
Morado *et al.*, 2005 [19]	Retrospective CH 50	<2% PUPs	NMA (Cut-off NA) Frequency NA	On demand patients: 78% Prophylaxis patients: 0%	Prophylaxis vs. On-demand: 0.04 (0.006–0.3)*	No inhibitor development in patients on prophylaxis Prophylaxis seems to protect against inhibitors
Santagostino *et al.*, 2005 [13]	CC 108	<2% PUPs, MTPs	BA and NMA (>0.5 twice) At least 1× per 3 months for first 100 EDs 1× per 6 months 100–200 EDs 1× per year thereafter	NA	Age at start: <11 month: 3.3 (0.9–12.0) 11–16 months: 2.5 (0.7–8.9) Prophylaxis: 0.2 (0.06–0.9)	Age at start not associated with a higher risk of inhibitors Prophylaxis seems to be protective against inhibitor formation
Gouw *et al.*, 2007a [15]	Retrospective CH 366	<2% PUPs	BA (Cut-off NA – according to local laboratory 0.6–1.0– and only clinically significant inhibitor) Frequency NA	24%	Age <1 month: 1.6 (0.6–4.1) Prophylaxis: 0.5 (0.2–0.9) Surgical procedures at start of treatment: 2.6 (1.3–5.1)	Age at first treatment was not associated with a higher risk of inhibitors after adjustment for confounding factors Regular prophylaxis was associ ated with a 60% lower risk than on demand treatment. Surgical procedures and peak treatment moments at start of treatment were associated with a higher risk of inhibitors
Chalmers *et al.*, 2007 [20]	Retrospective CH 348	<1% PUPs	BA (≥1.0 twice) ≥1× per 3–6 months	<1 month: 26% 1–6 months: 25% 6–12 months: 21% 12–18 months: 20% >18 months: 9%	Onset of treatment at age <1 month = 1.15 (CI 0.47–2.85) $P = 0.018$ across all age groups $P = 0.44$ at different time points during the 1st year of life	Exposure to FVIII during the neonatal period is not associated with a higher incidence of inhibitors

Table 2. *Continued*

Study	Type of study No. patients	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Inhibitor incidence	OR/RR	Results and conclusions
Kurnik *et al.*, 2009 [21]	Retrospective CH 56	<1% PUPs, PTPs	NA (Cut-off NA) Frequency NA	Standard prophylaxis (40–50 IU kg^{-1} × 3 weekly) 14/30 (47%) New prophylaxis (~25 IU kg^{-1} × 1 weekly) 1/26 (3.8%)	0.05 (0.001–0.37)	Early low-dose prophylaxis to avoid danger signals may reduce inhibitor risk

BA, Bethesda assay; BU, Bethesda units; CC, case–control study; CH, cohort study; CI, confidence interval; ED, exposure day; HA, haemophilia A; HB, haemophilia B; MTP, minimally treated patients; NMA, Nijmegen assay/modification; NA, not available; OR, odds ratio; PUP, previously untreated patients; pdFVIII, plasma derived FVIII; rFVIII, recombinant FVIII; RR, relative risk.

*Calculated by the current authors from data given in the cited study.

†Multivariate analysis of age at first FVIII exposure, calendar year at first exposure (before or after 1985) and baseline FVIII level (greater or less than 1 IU dL^{-1}).

Vaccinations, infections, extravascular infusions, blood components, concurrent immunological disorders

It has been postulated that challenges to the immune system (such as infections or vaccinations) or genetic factors involving immune response genes and cytokine production might influence inhibitor formation in patients with haemophilia [13, 22]. It has also been suggested that extensive tissue damage and inflammation may trigger an antibody response against extra-vascular FVIII [22].

Our search showed that there is a paucity of studies investigating these risk factors (Table 3) [13,16,23]. One case–control study [13] has investigated vaccination or infection in 60 patients and 48 control subjects. Infections were present, or vaccinations performed, during active FVIII treatment in 12 patients and 11 controls. Although no apparent association was found between vaccination or infection and the development of inhibitors, no conclusions could be drawn as this was a single study in which selection bias could not be ruled out. Similarly, only one study that considered blood components could be identified. This study [23] was difficult to evaluate, as the patient group was heterogeneous with respect to the type of blood products received.

In a recent case–control study, acute hepatitis was frequently reported within 4 months of inhibitor detection and did not occur at all in matched controls [16]. HBsAg positivity was associated with inhibitor development, suggesting a higher risk of inhibitors in association with infection. No association with vaccination was described.

Two studies addressed the initial exposure to blood components and inhibitor formation [16,23]. In the case–control study, inhibitor patients had received significantly more FVIII concentrates and less non-concentrate products than controls. No conclusions can, however, be drawn from these two studies, as the study groups were small, not well-defined and heterogenous with respect to the timing and type of blood products received.

Survey. With the exception of infections, clinical experience within the group would suggest that the majority rated these factors as of moderate to low importance (3–1) in inhibitor development (Fig. 1), and their influence on clinical practice was also moderate to low (Fig. 2). When rated on a scale of 0–100 there was a wide variation of opinion. Infection was the only parameter consistently reported to be of moderately high importance and impact clinical practice (Fig. 1).

Table 3. Influence of vaccinations, infections, extravascular infusions, blood component and concurrent immunological disorders on inhibitor production.

Study	Type of study No. patients	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Inhibitor incidence	OR/RR* (95% CI)	Results and conclusion
Yee *et al.*, 1999 [23]	Retrospective case series 431	All severities PUPs, PTPs	Biggs and Bidwell in earlier years BA >1979 (Cut-off NA) Once yearly until 1983 1× per 3–4 months after 1983	Severe: 10% Moderate: 4.4% 27/431 (6.3%) inhibitors 20 patients with inhibitors had received more than one type of blood component	NA	No specific data on blood components given and the majority of patients received several products before developing inhibitors. Low frequency of inhibitors suggesting that low titre and transient inhibitors may have been missed in the early period as a result of infrequency of inhibitor testing
Santagostino *et al.*, 2005 [13]	CC 108	<2% PUPs, MTPs	BA and NMA (>0.5 twice) 1× per 3 months for first 100 EDs 1× per 6 month 100–200 EDs 1× per year thereafter	NA	Infection and/or vaccination: 0.8 (0.3–2.1)	Infections/vaccinations in 20% of cases and 23% of controls – (no significance) FVIII infusion during vaccination or infection showed no role in inhibitor development
Ragni *et al.*, 2009 [16]	CC 77	All severities NA	NA (Cut-off NA) Frequency NA	NA	HBsAg+ 5.5 (1.2–25.4)* Initial product FVIII concentrate: 10.4 (2.9–37) Initial product Non-concentrate product: 0.09 (0.03–0.31)	Acute hepatitis within 4 months of inhibitor detection in 40.0% of inhibitor cases compared with 0% of controls ($P = 0.001$) No association with vaccination

BA, Bethesda assay; BU, Bethesda units; CC, case–control study; CH, cohort study; ED, exposure day; HA, haemophilia A; HB, haemophilia B; MTP, minimally treated patients NMA, Nijmegen assay/modification; NA, not available; OR, odds ratio; PUP, previously untreated patients; pdFVIII, plasma derived FVIII; rFVIII, recombinant FVIII; RR, relative risk.
*Calculated by the current authors from data given in the cited study paper.

Recommendation. There is insufficient evidence with which to make recommendations about the inhibitor risk associated with vaccinations, infections, extravascular infusions, blood components and concurrent immunological disorders.

Theoretically, however, exposure to the deficient factor in association with immune challenges like vaccination and infection could increase the risk. Therefore, while waiting for studies to be performed, the EHTSB recommended that vaccinations should preferentially be given subcutaneously, avoiding a concomitant infusion of a factor concentrate. In addition, whenever possible, replacement therapy should be avoided in association with severe infections and the exposure to all types of blood components minimized.

Intensity of treatment, surgery, major bleeds and continuous vs. bolus infusion

Severe bleeds and surgery are characterized by cell damage and the release of endogenous danger signals that could potentially promote inhibitor development [4]. Initially, haemostatic cover was achieved by the use of bolus injections (BI), but more recent studies have shown that continuous infusion (CI) is as an attractive alternative treatment modality in many patients [24]. The advantages of CI are that it avoids both deep, and potentially dangerous, troughs and unnecessarily high levels of the factor obtained with BI, thereby improving the cost-effectiveness [25]. However, concerns have been raised about a potential association between the use of CI and inhibitor development [26–29].

The literature review identified 19 full manuscripts in this category (Table 4) [13,15,24,27,29–43]: 14 were case series, three cohort studies and one case–control study. No studies solely evaluated severe bleeds and the risk of inhibitor development. Intensive treatment, in most instances initiated because of a surgical procedure, was examined in 14 studies which included a total of 412 treatments in 348 patients. Of these, 16 patients (4.6%) developed an inhibitor. All but one of the cases was reported in previously untreated patients (PUPs) or minimally treated patients (MTPs), i.e. patients at high risk of developing antibodies. Six of these patients were treated with BI and nine with CI. Among the 229 patients defined as PTPs, only one inhibitor case was reported. Generally speaking, evaluating CI vs. BI was not a simple task as most of the CI patients were subsequently treated with additional intensive BI therapy for several days or weeks. In addition, confounding factors were rarely considered in the investigations and the possibility of selection bias could not be excluded in the majority of cases.

The case–control study by Santagostino *et al.* [13] did not find a higher prevalence of surgery among inhibitor compared with non-inhibitor patients. By contrast, two retrospective studies of PUPs demonstrated that major surgery at any exposure day was associated with increased inhibitor risk [15,41].The association between inhibitor development and surgical procedures and/or peak treatment moments was even more pronounced if they occurred at the start of exposure to FVIII. Eckhardt *et al.* [43] reported an increased susceptibility of mild haemophilia A patients with the Arg593Cys genotype for inhibitor development after intensity of treatment for surgery, especially when continuous infusion was used [43].

Survey. Within the group, severe bleeds were rated as being of quite high importance by the majority of physicians, but the opinion on treatment intensity, surgery and continuous infusion were very variable (Figs 1 and 2). All of these factors had some influence on the clinical practice of members of the group.

Recommendation. European Haemophilia Therapy Standardisation Board recommended that prospective studies that primarily address the potential of intensive treatment (either with BI or CI), surgery and severity of bleeds as risk factors for inhibitor development are warranted. It is crucial to define a haemostatic minimum for particular clinical situations and to use treatment regimens of comparable intensity. Given the evidence in PUPs it is, however, desirable to minimize intensive treatment whenever possible to avoid treatment in association with immune system challenges. Available data do not support the concept that the use of CI *per se* in patients with severe haemophilia is associated with a higher risk of inhibitor development. This is further supported by the findings in an EHTSB study of continuous vs. bolus infusion in which only three of 659 patients (0.4%) with severe haemophilia developed inhibitors (Angelika Batorova personal communication, manuscript in progress). In the case of milder forms of haemophilia, the board recommends further thorough study.

Factor concentrates

The ability to provide effective replacement therapy has been a major achievement, and huge advances have been made in the production of different types of concentrates, ranging from cryoprecipitate to

Table 4. Effects of major bleeds, intensity of treatment, surgery and continuous vs. bolus infusion on inhibitor development.

Study	Type of study No. patients (No. procedures)	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Inhibitor incidence	OR/RR (95% confidence interval)	Results and conclusion
White *et al.*, 1997 [30]	Prospective CS 13 (24: all BI)	<5% PTPs	BA (0.6) 1× per month	0%	NA	No inhibitors developed after surgery
Berntorp *et al.*, 1997 [31]	Prospective CS 17 (22: all BI)	≤2% PTPs	BA (Cut-off NA) After 2 weeks and 1 month followed by 1× per 3 month	0%	NA	No inhibitors in PTPs undergoing surgery
Campbell *et al.*, 1998 [32]	Retrospective CH 17 (17: all CI)	All severities (<1%: 11) PTPs	BA (Cut-off NA) Frequency NA	0%	NA	No inhibitors observed with CI
Rochat *et al.*, 1999 [33]	Prospective CS 5 (5: all CI)	<1% PTPs	BA (Cut-off NA) Frequency NA	0%	NA	CI is a safe method for intensive therapy
Tagariello *et al.*, 1999 [34]	Prospective CS 15 (incl 1 HB) (15: all 15 CI)	All severities PTPs, MTPs	BA (Cut-off NA) Frequency NA	6.7% (mild, <20 EDs)	NA	CI is safe for patients undergoing major surgery
Batorova *et al.*, 2000 [24]	CS 40 (43: 25 CI, 18 BI)	<1% PTPs	BA (0.6) Frequency NA	0%	NA	No inhibitors in PTPs undergoing surgery with BI and CI
Scharrer *et al.*, 2000 [35]	Prospective CS 15 (22: all BI)	<2% PTPs	BA (≥0.6) Frequency NA	6.7%	NA	rFVIII is a safe method to use in patients undergoing surgery
Dingli *et al.*, 2002 [36]	Retrospective CS 28 (45; all CI)	All severities (<1%: 15) PTPs, MTPs	BA (0.6) Frequency NA	0%	NA	CI can be used safely in patients undergoing surgery
Ghosh *et al.*, 2002 [37]	Retrospective CS 35 (35 BI/CI not stated)	All severities (<1%: NA) PTPs, MTPs	BA (Cut-off NA) Postoperatively	17%	NA	Surgery and intense FVIII therapy may trigger inhibitor formation
Scharrer *et al.*, 2002 [38]	Prospective CS 22 (30: all BI)	≤2% PUPs, MTPs, PTPs	NA (Cut-off NA) Frequency NA	9.1%	NA	rFVIII is a safe method to use in patients undergoing surgery
Sharathkumar *et al.*, 2003 [27]	Retrospective CS 7 (16: 7 CI, 9 BI)	<40% PUPs, MTPs	NMA (Cut-off NA) Frequency NA	CI (± BI): 25% BI: 0%	NA	Intensive exposure to FVIII by CI may be associated with a higher risk of inhibitors in mild haemophilia
Mulcahy *et al.*, 2005 [39]	Retrospective CS 12 (18: all CI)	All severities (<1%: 3) PTPs, MTPs	BA (Cut-off NA) Frequency NA	8% (FVIII:C >5%)	NA	Intensive therapy in mild haemophilia is possibly a risk factor for inhibitor formation

Table 4. *Continued*

Study	Type of study No. patients (No. procedures)	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Inhibitor incidence	OR/RR (95% confidence interval)	Results and conclusion
Santagostino *et al.*, 2005 [13]	CC 108 (Surgical procedures in 26)	<2% PUPs, MTPs	BA and NMA (>0.5 twice) At least 1× per 3 months for first 100 EDs 1× per 6 month 100–200 EDs 1× per year thereafter	NA	Surgery (all): 1.1 (0.5–2.7)* ICH (all): 2.0 (0.37–10.1)*	No association between severe bleeds/surgery and inhibitors
von Auer *et al.*, 2005 [29]	Retrospective CS (250 : all CI)	All severities PUPs, PTPs	NA (Cut-off NA) Frequency NA	4.0%	NA	Five of 10 patients with inhibitors after the use of CI suffered from mild and moderate haemophilia A suggesting the existence of an unknown risk factor, related to CI, in these patients
Bidlingmaier *et al.*, 2006 [40]	Prospective CS 55 (55: 43 CI, 12 BI)	All severities (<1%: 38) PTPs, MTPs	BA (0.6) Frequency NA	3.6% (both <20 EDs and CI)	NA	CI is safe and effective method for perioperative care but should be used only in patients who are beyond 20 EDs
Gouw *et al.*, 2007a [15]	Retrospective CH 366 (80 surgical procedures and 251 peak treatments CI/BI NS)	<2% PUPs	BA (Cut-off NA – according to local laboratory 0.6–1.0– and only clinically significant inhibitor) Frequency NA	Reason for first treatment-Surgical procedures: 65% Peak treatment ≥5 days: 56%	Surgical procedures: 2.6 (CI 1.3–5.1)‡ Peak treatment ≥5 days: 3.1 (1.9–5.0)‡	Surgical procedures and peak treatments (≥5 days) at start of treatment were associated with a higher risk of inhibitors
Gouw *et al.*, 2007b [41]	Prospective CH 236 (44 surgical procedures and 31 peak treatments CI/BI NS)	<2% PUPs	BA (>0.6) 1× per 3 month	Reason for first treatment-Surgical procedures: NA Peak treatment ≥5 days: 53%	Surgical procedures: 2.7 (CI 1.3–5.7)‡ Peak treatment ≥5 days: 1.6 (0.9–2.8)‡	Peak treatment moments and surgical procedures were associated with a higher risk of inhibitors
Negrier *et al.*, 2008 [42]	Prospective CS 58 (65:18 CI, 47 BI)	≤2% PTPs	BA (Cut-off NA) 2 weeks after end of therapy	0%	NA	No inhibitors after intensive treatment rFVIII safe for use during surgery

Table 4. *Continued*

Study	Type of study No. patients (No. procedures)	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Inhibitor incidence	OR/RR (95% confidence interval)	Results and conclusion
Eckhardt *et al.*, 2009 [43]	Retrospective CH 138	2–40% NA	BA and NMA (1.0, or if <1.0 the FVIII ratio was ≤0.5 or spontaneous bleeds) ≥1 per year if FVIII was used during last 12 months	10%	Surgical procedures† 186 (2.5–1403)§ Use of CI during surgery: 13 (1.9–86)	The Arg593Cys genotype was identified in 8/10 patients with inhibitors. This mutation and intensive perioperative use of FVIII, especially when administered by CI, are associated with increased inhibitor risk in mild and moderate haemophilia A

C, Other factors or confounders; HA, haemophilia A; HB, haemophilia B; BA, Bethesda assay; BI, Bolus infusion; BU, Bethesda units; CC, case–control study; CH, cohort study; CS, case series; CI, continuous infusion; ED, exposure day; HA, haemophilia A; HB, haemophilia B; MTP, minimally treated patients; NMA, Nijmegen assay/modification; NA, not available; OR, odds ratio; PUP, previously untreated patients; pdFVIII, plasma derived FVIII; rFVIII, recombinant FVIII; RR, relative risk.

*Calculated by the current authors from data given in the cited study.

†First surgical procedure in a period of 3 months prior to inhibitor development.

‡Adjusted for baseline factor VIII:C, ethnicity, family history of inhibitors, age at first exposure and prophylaxis.

§Adjusted for Arg593Cys mutation, intensive FVIII treatment for surgery, continuous infusion, FVIII product change and family structure.

bioengineered recombinant products. Even though direct comparisons are lacking, it has been suggested that very high purity FVIII concentrates produced by monoclonal or recombinant technology are more antigenic than the older concentrates, resulting in increased risk of inhibitor development. In reviewing the pertinent literature, 26 relevant papers were identified: 11 case series and 15 prospective cohort studies. The number of participants ranged from 38 to 838 (Table 5) [1,20,23,44–66].

When comparing the immunogenicity of plasma-derived clotting products to those obtained by recombinant DNA technology, there was no consistent agreement in the literature. In addition, numerous concerns can be raised about the quality of the studies. They were primarily retrospective in design, the populations were not homogenous, patients were treated with a large variety of products, the methodologies were variable, and follow-up was sometimes insufficient. In addition, selection bias could not be ruled out in the majority of studies, and confounding factors were not addressed.

Apart from a few outbreaks of inhibitors caused by a change in the manufacturing process, studies of PTPs involving more than a thousand patients describe an incidence of inhibitors ranging from 0.9% to 2.9%. This clearly indicates that product immunogenicity and switching to a different product carry with them only a small risk for inhibitor development. In addition, PTPs are likely to be older than untreated patients, and other confounding and potentially contributory factors not considered will have, in some cases, an immunological impact.

The incidence of inhibitors in PUPs and MTPs with haemophilia A ranged from 4.4% [23] to 52% [1]. As a result of the potential influence of confounding factors, both genetic and non-genetic, it is not possible to fully appreciate the impact of the type of concentrate and product immunogenicity *per se*. It is also noteworthy that the incidence of inhibitors varies between cohorts despite the use of the same product, which underscores both the heterogeneity of the studies and the importance of a well-characterized cohort for study to better appreciate the immunogenicity of the product itself.

Survey. The issue of product switching was considered to be of moderate to low (3–2) importance and influence on clinical practice by the majority of the group. The type of product was considered of moderate to low importance (no individual rated it at 5) (Figs 1 and 2), but its influence on clinical practice was highly variable (Fig. 1).

Table 5. Effect of type of factor concentrate on inhibitor development.

Study	Type of study No. patients	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Type of product (No. patients)	Inhibitor incidence	OR/RR (95% confidence interval)	Results and conclusions
Lusher *et al.*, 1990 [44]	Prospective CS 38	≤5% PUPs, PTPs (cryo-precipitate)	NA (Cut-off NA) Frequency NA	pdFVIII	15.8%	NA	The study group is too small to draw any clear conclusions about the immunogenicity of the product
Ehrenforth *et al.*, 1992 [1]	Prospective CH 63	≤5% MTPs	BA (0.3) ≥1× per 20th ED	pdFVIII and rFVIII	All HA: 24% Severe HA: 52%	NA	The previous reports may underestimate the incidence of inhibitors in HA and the effect of new generation products in the inhibitor development are unclear
Addiego *et al.*, 1992 [45]	Prospective CS 184	<5% PUPs, MTPs, PTPs	BA (Cut-off NA) 1× per 3 months	pdFVIII	12.5%	NA	No significant adverse effect observed with an immunoaffinity purified concentrate
Addiego *et al.*, 1993 [46]	Retrospective CH 89	<1% PUPs	BA (Cut-off NA) 1–2× per year	cryoprecipitate and pdFVIII	28%	NA	Frequency of inhibitor development in severe haemophilia treated from birth with low or intermediate purity products may be greater than previously suspected and not lower than with recombinant products
Lusher *et al.*, 1993 [47]	Prospective CS 95	All severities PUPs	BA (≥0.6) 1× per 3 month	rFVIII	19.7%	NA	Benefits of rFVIII seem to outweigh the risks and transient inhibitors my represent part of the natural history of treatment
Rosendaal *et al.*, 1993 [48]	Retrospective CH1 447 Retrospective CH 2 144	<40% PTPs	BA (>1.0) Frequency NA	pdFVIII (switch)	Incidence before: 3.9–4.4/1000 pat years After: 20.1/1000 pat years 4.5 (1.4–14.3)	RR for exposure to FVIII CPS-P 1.24 (0.17–8.9)* Based on period 1	An increase in inhibitor development associated with switching from a particular pd FVIII product to another (FVIII CPS-P)
De Biasi *et al.*, 1994 [49]	Prospective CH 64	<5% PUPs	BA (≥0.8) ≥1× per year	pd intermediate purity FVIII (57) pd high purity FVIII (7)	Type of pd product: intermediate purity: 21% high purity: 14%	1.5 (0.2–9.7)	Inhibitor risk may be underestimated in previous studies and more data are needed to evaluate the antigenicity of very-high purity FVIII concentrates
Bray *et al.*, 1994 [50]	Prospective CS 71	≤2% PUPs	BA (Cut-off NA) 1× per 3 months	Fullength rFVIII	24%		Immunogenicity of rFVIII similar to pdFVIII Relatively high frequency of low titre inhibitor, many of which are transient

Table 5. *Continued*

Study	Type of study No. patients	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Type of product (No. patients)	Inhibitor incidence	OR/RR (95% confidence interval)	Results and conclusions
Giles *et al.*, 1998 [51]	Prospective CS 478 for 1 year; 338 for 2 years	NA PTPs	BA (≥0.5) ≥1× per year	rFVIII	3.8% year 1; 5.0% year 2 (2–3% over 2 years) 5% within 1 year after switch	NA	Switch to rFVIII previously treated with plasma derived products was not associated with an increase in FVIII inhibitor development
Yee *et al.*, 1999 [23]	Retrospective CH 431	All severities PUPs, PTPs	Biggs and Bidwell in earlier years BA >1979 (Cut-off NA) Once yearly until 1983 1× per 3–4 months after 1983 in PUPs	Whole blood, plasma, cryoprecipitate, pdFVIII, rFVIII, porcine FVIII	Severe: 10% Moderate: 4.4% 20 patients with inhibitors had received more than one type of blood component	NA	Low frequency of inhibitors suggesting that low titre and transient inhibitors may have been missed in the early period because of infrequency of inhibitor testing
Courter & Bedrosian, 2001 a[52]	Prospective CS 101	<2% PUPs	BA and NMA (0.6) 1× per 3 month	BDD rFVIII	32%	NA	The inhibitor risk with BDD FVIII comparable with that of full-length FVIII
Courter & Bedrosian, 2001b [53]	Prospective CS 116	<2% PTPs	BA and NMA (>0.6) Frequency NA	BDD rFVIII	0.9%	NA	Inhibitor development with BDD FVIII is similar to other recombinant FVIII products
Knobe *et al.*, 2002b [54]	Retrospective CH 116	<1% PUPs	NMA (Cut-off NA) ≥2× per year	Pd FVIII and rFVIII	HA: 19% HB: 37% Treated 1980–89 (mainly pdFVIII): 17% Treated 1990–99 (mainly rFVIII): 21%	NA	No association between the type of concentrate and inhibitor development
Kreuz *et al.*, 2002 [55]	Prospective CH 72	≤5% PUPs	Modified BA (0.6) 1× per 3rd to 5th ED for the first 20 ED 1× per 10th ED until 200 ED then 1× per 3 months	pdFVIII (51) rFVIII (21)	pdFVIII: 37% rFVIII: 36%	NA	Groups are small and no statistically reliable statement can be made concerning concentrate and inhibitor development

Table 5. *Continued*

Study	Type of study No. patients	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Type of product (No. patients)	Inhibitor incidence	OR/RR (95% confidence interval)	Results and conclusions
Kreuz *et al.*, 2003 [56]	Prospective CH 156	≤5% PUPs	Modified BA (0.6) 1× per 3rd to 5th ED for the first 20 ED 1× per 10th ED until 200 ED then 1× per 3 months	HA -pd FVIII: (54) rFVIII (74) HB -pd FIX: (27) rFIX (1)	Haemophilia A -pdFVIII: 28% (Severe) 0% (Moderate) 22% (All) rFVIII: 40% (Severe) 17% (Moderate) 32% (All) Haemophilia B – 7.1%	NA	No significant differences in inhibitor incidence observed for pdFVIII and rFVIII
Lusher *et al.*, 2003 [57]	Prospective CS 218	<2% PUPs, PTPs	BA and NMA (≥0.6) 1× per 3 months	BDD rFVIII	PUPs: 31.7% PTPs: 0.9%	NA	Inhibitor development similar to that of full-length rFVIII and pdFVIII.
Yoshioka *et al.*, 2003 [58]	Prospective CS 43	All severities PUPs	BA (≥0.5) 1st year every 3 months then 1× per 6 months	Fullength rFVIII	34.9%	NA	A relatively high inhibitor incidence but rFVIII is effective and safe for the treatment of PUPs
Kreuz *et al.*, 2005 [59]	Prospective CS 61	<2% PUPs, MTPs	NMA (>0.6) 1× per 3rd to 5th ED for first 20 ED 1× 10th ED until 200 EDs then 1× per 3 month	rFVIII	15%	NA	Parameters other than the type of product of importance for inhibitor development
Kreuz *et al.*, 2006 [60]	Prospective CH 324 (including 46 HB)	All severities PUPs	NMA (>0.6) 1× per 3rd to 4th ED for first 20 EDs 1× per 10th ED until the 200 ED then 1× per 3 month	rFVIII (95) pdFVIII (88)	rFVIII: 36% pd FVIII: 21% $P = 0.08$; Fisher's exact test)	NA	So far no significant difference in inhibitor incidence between type of concentrate
Goudemand *et al.*, 2006 [61]	Retrospective CH 148	<1% PUPs	BA (≥0.6) Frequency NA	pd FVIII rFVIII	pd FVIII: 10.3% rFVIII: 32.3%	Adjusted relative risk (all inhibitors): 2.4 (1.0–5.8)	The risk of inhibitor development was higher in patients treated with rFVIII than in patients treated with pdFVIII

Table 5. *Continued*

Study	Type of study No. patients	Severity and previous treatment	Inhibitor test (Cut-off level BU mL^{-1}) Frequency of testing	Type of product (No. patients)	Inhibitor incidence	OR/RR (95% confidence interval)	Results and conclusions
Gringeri *et al.*, 2006 [62]	Retropective CS 99	≤5% PUPs (31), MTPs (68)	BA (>0.6) Every 5–10 ED for 3–6 months	pd high purity FVIII with VWF	7.1%	NA	Inhibitor incidence is low and similar to other pd FVIII containing VWF
Kempton *et al.*, 2006 [63]	Retrospective CH 838	All severities PTPs	BA (>0.5) Frequency NA	pdFVIII (203) rFVIII (554)	pdFVIII: 1.0% rFVIII: 0.9%	0.9 (0.2–4.7)*	Inhibitor incidence is low in PTPs and small non-randomized studies are therefore inadequate to determine the inhibitor development after exposure to a novel product
Chalmers *et al.*, 2007 [20]	Retrospective CH 348	≤1% PUPs	BA (≥1.0 twice) ≥1× per 3–6 months	pdFVIII (132) rFVIII (172)	rFVIII: 27% pdFVIII; 14% 2.0 (1.2–3.3)*	Multivariate analysis: 1.8 (0.9–3.7)	Initial treatment with rFVIII associated with a higher incidence of inhibitors than with pdFVIII
Gouw *et al.*, 2007c [64]	Retrospective CH 316 pts	<2% PUPs	BA (Cut-off NA – according to local laboratory 0.6–1.0 – and only clinically significant inhibitor) Frequency NA	pdFVIII (135) rFVIII (181)	pdFVIII: 21.5% rFVIII: 29.3%	Plasma derived adjusted RR 0.7 (0.4–1.1)†	RFVIII and switching FVIII products are not associated with a higher risk of inhibitors. FVIII with high VWF was not associated with a lower inhibitor risk
Delumeau *et al.*, 2008 [65]	Prospective CHS (PMS) 613	All severities MTPs, PTPs	NA (Cut-off NA) Frequency NA	Full-length rFVIII	0.8%		Incidence of inhibitors comparable with other studies
Musso *et al.*, 2008 [66]	Prospective CS (PMS) 212	<2% PUPs, MTPs	NA (Cut-off NA) Frequency NA	Full-length rFVIII	2.8% (including recurrent inhibitors)	NA	Low incidence of inhibitors comparable with other studies

BA, Bethesda assay; BI, Bolus infusion; BU, Bethesda units; CC, case–control study; CH, cohort study; CPS-P, controlled-pore silica adsorbed and pasteurised; ED, exposure day; HA, haemophilia A; HB, haemophilia B; MTP, minimally treated patients; NA, not available; NMA, Nijmegen assay/modification; NS, not stated; OR, odds ratio; pd, plasma derived; pdFVIII, plasma derived FVIII; PMS, post-marketing surveillance study; PUP, previously untreated patients; rFVIII, recombinant FVIII; RR, relative risk; VWF, von Willebrand factor.

*Calculated by the current authors from data given in the cited study.

†Adjusted for baseline factor VIII activity level, ethnicity, factor VIII gene mutation type, age at first exposure, duration between exposure days, dose of factor VIII, and regular prophylaxis.

Recommendations. The European Haemophilia Therapy Standardisation Board concluded that in PTPs there is no evidence to suggest that the immunogenicity of various types of product will differ and that the use of these concentrates, or a switch between them, will be associated with a risk of inhibitor development. Thus far, there is insufficient evidence with regard to inhibitor risk for a treating physician to select one product over another and recent findings suggesting an impact of the FVIII polymorphism on inhibitor risk require further studies [67]. Evaluating whether the type of concentrate has the ability to modulate the risk in PUPs in a significant way and thereby establishing implications for the use of different types of factor concentrates will require well-designed, prospective clinical trials. These trials must also consider all other aspects of product choice. Independent of the concentrate used, EHTSB recommended that all patients should be carefully monitored during the high-risk period at start of treatment.

Discussion

This review of the literature revealed a lack of data allowing a proper appreciation of the potential impact of a variety of non-genetic risk-factors on inhibitor development. The most important factors appear to be: the reason for the first infusion at young age and the intensity of treatment. In these situations the immune system may be exposed to the deficient factor within the context of immune system challenges and the occurrence of danger signal(s). The prophylactic use of factor concentrates to prevent bleeds is state-of-the-art. However, recent data also suggest that prophylaxis might modulate the immune response to treatment when started at young age and thereby reduce inhibitor risk. The latter hypothesis requires more investigation, which is also the case for understanding the optimal dosing required to allow this potential benefit of prophylaxis to occur. For most of the other debated non-genetic factors, the impact on the immunological outcome is, to date, not supported by the literature. Because the factors are often interrelated, it is also difficult to identify the relative contribution of each. This is also reflected by the results of the survey carried out among the EHTSB members, in which the impact of the majority of the factors was extremely variable; a pattern also recently reported in a survey by van den Berg and Chalmers [68]. The genetic profile of the patient will have a major impact on the immunological outcome and must be considered. This has not been done in the current literature. As haemophilia is a rare disease, and inhibitors develop in a minority of patients, the statistical power of studies addressing these issues will, by definition, be limited. In light of the complexity of the aetiology of inhibitor development, future research should be directed at the identification of early immunological markers of high risk patients.

In 2007, the EMEA [8] produced a report that defined many of the variables that should be considered when evaluating the literature on inhibitor formation. Unfortunately, several of these variables have not been included in a substantial number of published studies, which will indeed influence the accuracy, validity and interpretation of the data. For example, the type of assay used to measure and to identify the inhibitor. The Nijmegen modification of the Bethesda assay was considered the 'gold standard' with a cut-off point of >0.6 BU. In addition, confirmatory tests on a second, separately drawn sample within a month should be performed. As seen in the tables, however, these requirements are frequently not adhered to by studies published in the current literature. Moreover, the previous exposure to factor concentrates will be of major importance. According to the EMEA report, PUPs should be defined as those patients who have never been exposed to clotting factor products. Frequently, inhibitor studies involve patients who are considered to be MTPs. This term was considered inappropriate and these patients should instead be defined as previously treated patients (PTPs). This will have an impact on the interpretation of inhibitor incidence in each cohort described. It was also suggested that the number of EDs should be utilized as parameters to categorize risk rather than rely on the categories of PUP or MTP. In the case of factor concentrate immunogenicity, it was agreed that PTPs was the optimal group to study to limit the impact of confounding factors. Furthermore, all studies should ideally provide patients' characteristics, including severity of the disease, age at first exposure, race and ethnicity, type of gene mutation, family history of inhibitors, general health status, reason for treatment, type of regimen and intensity of treatment, EDs, surgery, infection and vaccination. Most of this information is not available in the studies performed to date.

Regarding future investigations in the area of inhibitor development, EHTSB recommends that the studies be carried out on well characterized, large cohorts of severe (clotting activity <1%), infusion-naïve PUPs with consecutive enrolment. The only exception to this recommendation is the evaluation of immunogenicity of new factor concen-

trates which, according to the EMEA guidelines, should first be carried out in PTPs. Potentially confounding factors should be addressed and genetic factors taken into account. Validated assays (e.g. Nijmegen) for inhibitor analysis should preferably be performed in a central laboratory with a pre-defined cut-off value and, in a case where an inhibitor is detected, confirmed with another test within the shortest possible interval. Patients who develop an inhibitor should be classified by clear criteria as high responders (≥5 BU), low responders (<5 BU) and whether the inhibitor is transient (disappearing within 3 months without a change in treatment regimen, or disappearing) or not. Enzyme linked immune sorbent assay (ELISA) should also be performed to detect all antibodies produced against the deficient factor.

Well-conducted studies will contribute to our understanding of the pathophysiology of inhibitor development, thereby enabling the use of treatment approaches with the potential to minimize inhibitor development in patients with haemophilia.

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